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Transport and emissions of methane through stems of boreal trees in controlled condition

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Tiivistelmä – Referat – Abstract

Greenhouse gases are essential in controlling the surface temperature of the Earth. Methane is one of the most abundant greenhouse gases in the atmosphere. it has an important role in the atmospheric chemical processes, and its atmospheric concentration has increased dramatically from pre-industrial time. In 2006, studies revealed that terrestrial plants are capable of emitting methane under aerobic conditions which led to the conclusion that the contribution of forests to the global methane budget needs to be considered.

In my thesis the aim was to assess the capacity of boreal tree stems to transport methane, to quantify the radial diffusivity of methane in the stem of different tree species and evaluate the effects of various factors on regulating stem gas transport. Gas transport of Scots pines (*Pinus sylvestris*) and Birches (*Betula pubescens*) tree stems were examined in the laboratory under controlled conditions.

The results highlighted that birch stem samples have a higher methane stem fluxes compared to pine samples. The result also indicated that birches accumulated less methane inside the stem compare with pine samples.

One of the most significant findings from this study is that birch stem samples have the higher average methane and carbon dioxide diffusivity compared to pine samples. This finding also explains the smaller accumulated methane gas inside the birch stems compared to pine stems. Also, the differences in the diffusivity may result from differences in the anatomical composition of these tree species, including heartwood, sapwood, bark tissue and lenticel densities.

Avainsanat – Nyckelord – Keywords
Methane, Boreal forest, Diffusivity, Greenhouse gases, Chamber measurements, *Pinus sylvestris, Betula pubescens*

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Muita tietoja – Övriga uppgifter – Additional information

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1. Introduction

Greenhouse gases (GHGs) are constituted of gaseous molecules with the attribute of absorbing and re-emitting thermal infrared solar radiation in the lower atmosphere of the Earth. Their impact influences the Earth's climate considerably. Carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone-depleting substances (ODSs) and Sulphur hexafluoride (SF_6) lead into the atmosphere due to natural processes or anthropogenic activities and their impact might affect the climate from decades to thousands years. Radiative efficiency and lifetime of an emitted greenhouse gas evaluate its climate effect and global warming potential (Montzka et al. 2011). Greenhouse gases are essential to control the surface temperature of the Earth. Since the pre-industrial era, the average temperature of the Earth has raised due to the increasing the major greenhouse gases global emission. The Intergovernmental panel on climate change (IPCC) reported the significant increase in the global emission of GHGs by 70 percent, from 1970 to 2004 (IPCC, 2007). Since late 1800s, the surface temperature of Earth raised by 0.6° Celsius (Steinfeld, 2006). Accordingly, by continuing the current rate the global warming will reach 1.5° by 2030-2052 (IPCC, 2018).

It has been estimated that CO_2 as one of the most abundant greenhouse gases is responsible for about two thirds of the predicted global warming (Papadimitriou, 2004). According to report of IPCC (2013) the greenhouse gases concentration exceeded the unprecedented level over the last eight hundred thousand years. The subsequent global warming has changed the atmospheric weather generating mechanism by increasing the quantity of water and energy into the hydrological cycle. Industrialization and anthropogenic climate change influence on the global carbon cycle. Carbon cycle features the movement of carbon among the atmosphere, ecosystem, soil and hydrosphere of the Earth. Over decades, human activities including burning fossil fuels have interrupted the global carbon budget considerably (Churkina,2016).

Forests are the considerable component of global carbon cycle (e.g. Canadell & Raupach, 2008). In recent years, forest ecosystem methane researches have denoted trees are as important as forest soil in the forest methane budget (e.g. Covey& Megonigal, 2018). Methane dynamics in the forest is consisted of a complex combination of plants, microbial and abiotic process related to the living and dead trees. Presence of plants in vegetated regions increased methane transport 10 times compared to the non-vegetated areas. The results denoted that 90% of methane emission was emitted through the plants (Whiting & Chanton,1992). Machacova et al. 2016 stated mature Scot pine trees frequently emit methane from the stem and shoots, and higher soil water content increases the methane emission from the boreal forest trees, considerably.

Several lines of evidence suggest that the gas transport within trees mostly occurs via radial diffusion (Teskey et al, 2008). Diffusivity specifies the rate of the gas exchange along with the concentration gradient. It has been noted that some of the CO_2 produced by respiring cells in the tree, diffuse into the atmosphere directly. Although various barriers in the inner bark and xylem cause the building up high concentration carbon dioxide in the tree stem (Teskey et al, 2007).

2. Background and theory

2.1 Sources and sinks of methane and global methane budget

Methane is a long-lived greenhouse gas with considerable warming potential and its atmospheric concentration has increased exponentially from pre-industrial time (Lelieveld et al., 1997). As one of the most abundant greenhouse gases in the atmosphere, the oxidized methane controls important chemical activities in the

Table 1. methane emission classified by source of production (Adapted from The Global Methane Budget 2000-2012 (2016). (*) demonstrates natural fluxes, (O) anthropogenic fluxes and (X) natural and anthropogenic.

Global methane budget	
Sources (Total emission ~ 558 Tg/year)	**Sinks** (Total sink ~ 548 Tg/year)
O **Fossil fuel** (105 Tg/year) Production and use	**Sink from chemical reactions in the atmosphere** (515 Tg/year)
O **Agriculture and waste** (188 Tg/year)	
X **Biomass burning** (34 Tg/year)	**Sink in soil** (33 Tg/year)
* **Wetlands** (167 Tg/year)	
* **Other natural emissions** (64 Tg/year) Geological, termites, oceans, forests, lakes, permafrost	

tropospheric and stratospheric levels. The interaction between sources and sinks substantiates the global methane budget. To illuminate the uncertain areas, the global budget has been investigated for decades (Saunois et al., 2016).

There are three main processes to define methane formation: Biogenically due to the degradation of organic matter under anaerobic conditions, thermogenically during the slow transformation of organic matter to the fossil fuel and pyrogenically through the incomplete combustion of organic matter (Kirschke et al, 2013).

The type of methane emission via different processes is characterized by its isotopic composition. In general, the methane emission sources are divided into the natural and anthropogenic sources. Table 1 demonstrates the global methane budget

including sinks and sources (The global methane budget 2000-2012, 2016). Table denoted that wetlands have the most significant influence in the global methane budget, annually (~ 167 Tg per year). In addition, the temperature of soil affects the changing rate of methane production in the wetlands (Potter et al, 2006). In contrast, the main sinks of methane in the atmosphere are oxidation reaction with hydroxyl (OH) radicals in the troposphere (Kirschke et al, 2013) and bacterial oxidation of methane in the soil (Le Mer and Roger, 2001) which oxidizes a significant volume of soil derived methane to carbon dioxide.

GK Heilig 1994 concluded where human impact does not perform the direct role in the atmospheric methane destruction, while the atmospheric pollutant is able to decrease the OH radical level and hence indirectly affect the methane lifetime in the atmosphere, respectively. It is conceivable that the atmospheric methane controls the radiative balance of Earth by its oxidation involving into the production of the major greenhouse gases such as CO_2 and water vapor, contributing to the global warming via its infrared absorption spectrum and dominating the lifetime of critical climatic gases including ozone (GK Heilig , 1994).

2.2 Methane cycling process in forests

Wetlands, rice paddies, animals, fossil fuel and biomass burning are identified as the major sources of methane production. The total global methane emission is estimated between 500 to 600 Tg[1] year (Watanabe, 2012). Keppler et al. (2006) first revealed that terrestrial plants are capable of emitting methane under aerobic conditions. This was supported by evidence from the observation of the methane emissions over tropical evergreen forests which could not be delved into the current global methane budget. The biological processes happening in soil and soil surface

[1] 10^{12} grams

are the main sources of methane emission into the atmosphere (Dalal, 2008). In line with previous studies, forests are considered as the source of methane due to their soil activities. Also, they have been identified as important sinks for greenhouse gases. Keppler's analysis found evidence for methane formation in plants by an obscure process under oxic conditions. The results lead to the conclusion that the contribution of forests to the global methane budget needs to be adjusted.
This result might affect the policies related afforestation, reforestation and deforestation activities and forest management protocols require to be reevaluated. Keppler's outcome suggested the methane emission by plants could make more significant contribution to the atmosphere during pre-industrial times, when the role of anthropogenic sources was negligible.

2.3 Methane, forest soil and vegetation

Soils are considered as significant sinks and sources of greenhouse gases. Soils are responsible for 45 percent of total global methane production. Regarding carbon cycle in the soil, methane is the most reduced form of the carbon and CO_2 is the most oxidized form in the cycle (Topp & Pattey, 1997). Analysis provides evidence that methane oxidizers (methanotrophs) and methane consumers are consisted of existing aerobic microbes in the soil that take O_2 to oxidize methane ($CH_4 + 2\,O_2 \rightarrow CO_2 + 2H_2O$, $\Delta G^\circ = -818\ \mathrm{kJ\ mol^{-1}}$)(Dalal, 2008). ΔG° refers to the standard free - energy change of the reaction. Figure 1 demonstrates the methane sink and production process in the soil. Soils produce methane by methanogenesis in anaerobic conditions. Methanotrophic microorganisms utilize methane and O_2 for their metabolic processes under the aerobic conditions (Dutaur & Verchot, 2007).

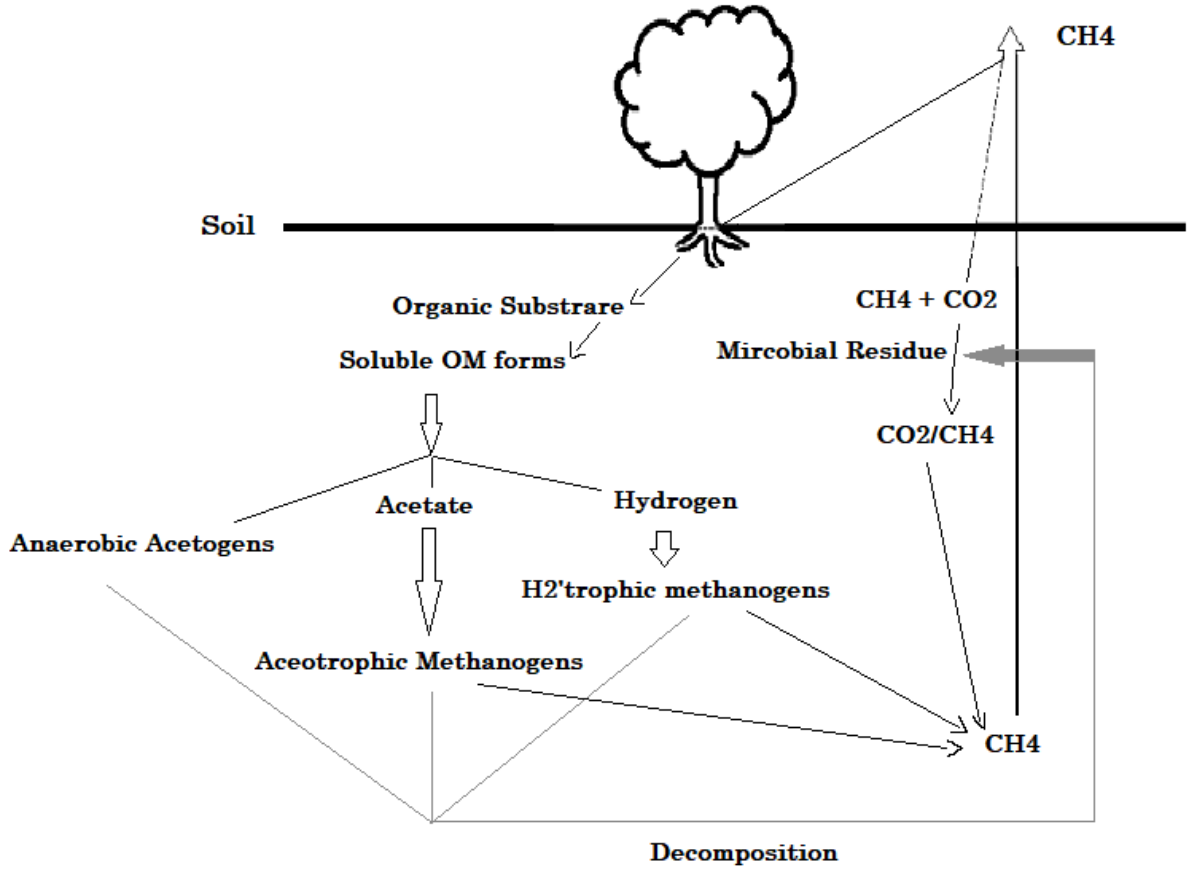

Figure 1 . Methane production and consumption in the soil.

(adapted from Dalal, 2008)

Reports have indicated that temperate forest soils have the highest rate of atmospheric methane consumption; -4.8±0.6 kg methane per hectare per year. A series of recent studies have revealed the forest vegetation composition, soil moisture content and soil pH are the effective factors controlling methane oxidation rates in the forest (Borken et al,2003;2006). Prior studies concluded that tree net production rate and methane emission are associated in the forest methane production cycle (Kepler et al, 2006).

Various parameters might affect the methane emission rate including the temperature, light, the physiology of the leaves and their link to the chemical composition of the biomass (Kirschbaum et al, 2007). Studies denoted methane emission from the tropical forest (Pangala et al, 2017; Pangala et al, 2012) due to the higher temperature and higher observed fluxes, consequently. Although related researches regarding methane emission from boreal forests demonstrated the similar processes but due to the lower temperature, with lower emission rates (0.005 $\mu g m^{-2} h^{-1}$) (Machacova et al. 2016).

2.4 Tree physiology

In general, a woody plant with a trunk over 4-meter tall is considered a tree (Siddiqui & Noman, 2017). Trees are vascular plants including three main parts: roots, trunk (stem) and crown. Roots are supporting the body of the plant and absorbing the required substances from the soil. The main part of the tree is configured as the stem (trunk) which is consisted of layers including inner bark (phloem), outer bark, cambium, sapwood (xylem) and heartwood (Figure 2).

Trees are categorized as two main groups, Gymnosperms (evergreens) or softwoods such as *Pinus sylvestris*, and Angiosperms or hardwoods including *Betula pubescens*. Phloem, which is composed of cells, transports the photosynthesis products where they are used or stored. Wood is chemically made up of carbon, oxygen and organic compounds (Siddiqui & Noman,2017). The contribution of the organic components differs between hardwood and softwood groups of trees.

Roots absorb water and minerals from the soil. Water is transported to the leaves by xylem. The conductive cells of xylem flow up the solution to the trunk, branches and leaves to be used in the photosynthesis process or transpiration (Kozlowski & Kramer,1979). Less than 5% of absorbed water remains inside the plant for growing process. The outer bark layer is considered as the protective and covering layer of the tree. Sap water streams through the xylem and flowing down in the inner bark or phloem.

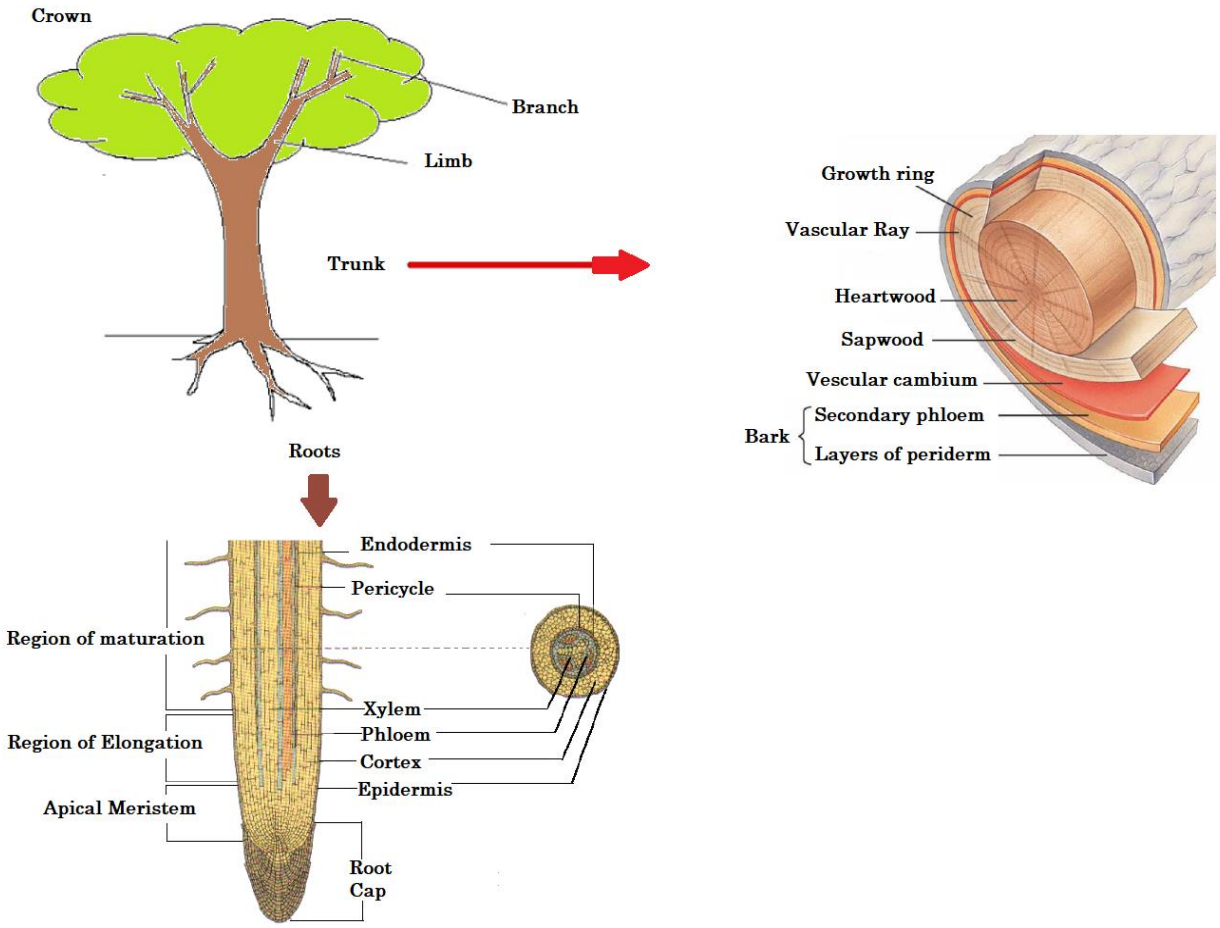

Figure 2. Anatomy of the tree, adapted from (Siddiqui & Noman, 2017)

Including the sections of tree roots and cross section of the tree trunk.

In plants, water is evaporating from the leaf cells surface to the air, consistently. Plant replaces the losing water by absorbing water from the soil. Theory of Sap Ascent in plants explains the process of upward movements of water through the plant from the soil to the leaf cell surfaces. Different water potential between the soil and the surrounding atmosphere of plant creates a gradient to force water moves through the drier areas (driving force). The drier surrounding air causes the more significant driving force and faster transpiration rate, consequently (Sterling, 2004). Transpiration is also facilitating gas exchanging between the leaves and atmosphere. In general, water uptake is a pivotal process for plants as it is a driving factor of biochemical processes.

Photosynthesis and transpiration are physiological processes that are bonded directly with water absorption by the plant. Soil moisture, aeration, temperature, the concentration of the soil solution, transpiration and root growth are regulating factors in the water absorption process by the tree roots.

The mechanisms of methane transport through the tree are not fully understood. Most of the methane transported through the tree stem and not via transpiration. Kutschera et al. 2016 suggested that various transport mechanisms involve in the methane transportation through the stem and domination of these mechanisms are related to the temperature. Low flux and low temperature lead to a substantial fractionation between emitted methane and root water, also higher flux and temperature with little or no fraction (Kutschera et al., 2016).

2.5 Aims and Objectives

Research on the global methane budget has a long tradition., although describing the role of boreal forest trees is a new approach. In this project, these questions have received substantial interest:

- How to describe the capacity of the tree stem for methane transportation?
- How might the wood anatomy of different species influence the capacity of methane transportation through the stem?
- How does anatomical variation direct the diffusivity in different species?
- How water content and the water flow rate might regulate the stem transportation of methane?

This study aims to provide evidence to illuminate the uncharted area regarding the addressed questions. The ultimate goal is to develop more sophisticated methods for estimating the tree stem capacity of methane production and transportation.

3. Methods and Experimental design

3.1 Sampling

This designed laboratory work was employed to support a series of experiments including the field, laboratory tasks and modelling regarding to study methane production, transport and emission from trees in the boreal forests. The examined samples consist of Scots pines (*Pinus sylvestris*) and Birches (*Betula pubescens*) which were cut down during November of 2016 from the vicinity of SMEAR II station (Station for Measuring Ecosystem-Atmosphere Relations) at Hyytiälä, Finland. The Hyytiälä field station SMEAR II (61º 51' N, 24°17' E, 181 m above sea level) is the research station of the institute for atmospheric and Earth system research (INAR) University of Helsinki (Hari & Kulmala, 2005).

At sampling, three pieces from three different tree heights of the stem were cut from each of the selected tree. The blocks were labeled by numbers which identify the number of the tree and height of the trunk. Upon arrival at the laboratory, the samples were stored in plastic bags in the laboratory freezer (−20°C) before the analysis.

3.2 Chamber setups

The setup of the chamber for measuring the emission of methane and CO_2 from the stem of the block mainly consisted of the chamber and uninterrupted flowing of solution through the block (Figure 3). The setup of chamber measurement is designed to detect CO_2 and CH_4 fluxes from the trunk of the block by following a defined protocol. Height and material of the built chambers are similar for all of the tested blocks from different species. More information related to the used material in the chamber setup are shown in Table 2.

Table 2 . Material of the configuration of chamber

Material	Location	Quantity
Neopren ring	Head/Bottom of the block	2
Clear blown cellophane stretch wrap	Main Body of chamber	Varies for samples
Metal bars	Poles of the body (Frames)	4
Plexiglas	Head/Bottom of chamber	2
Air circulating fan	Sides of the chamber (180^{o})	2

The diameter of the tree block varies, while the height of the sample required to be kept constant (= 28 cm) for all blocks. Hence, we cut samples into the mentioned height before the beginning of the experiment. Cutting the samples were done so that the block was kept under water to avoid embolism of wood cells.

We fixed the block between two pieces of Plexiglas. To prevent leaking, the head and bottom of the block were sealed by Neopren rings. A tank (= 20 liter) with a tap was filled with water-KCl (potassium chloride) mixture. Clear PVC (Polyvinylchloride) flexible tubing conducted solution from the tank into the measurement system (Figure 4). We employed clear blown cellophane stretch wrap to cover the surrounding area of the block. Four metal bars were used to support the wrapped cover around the sampling block.

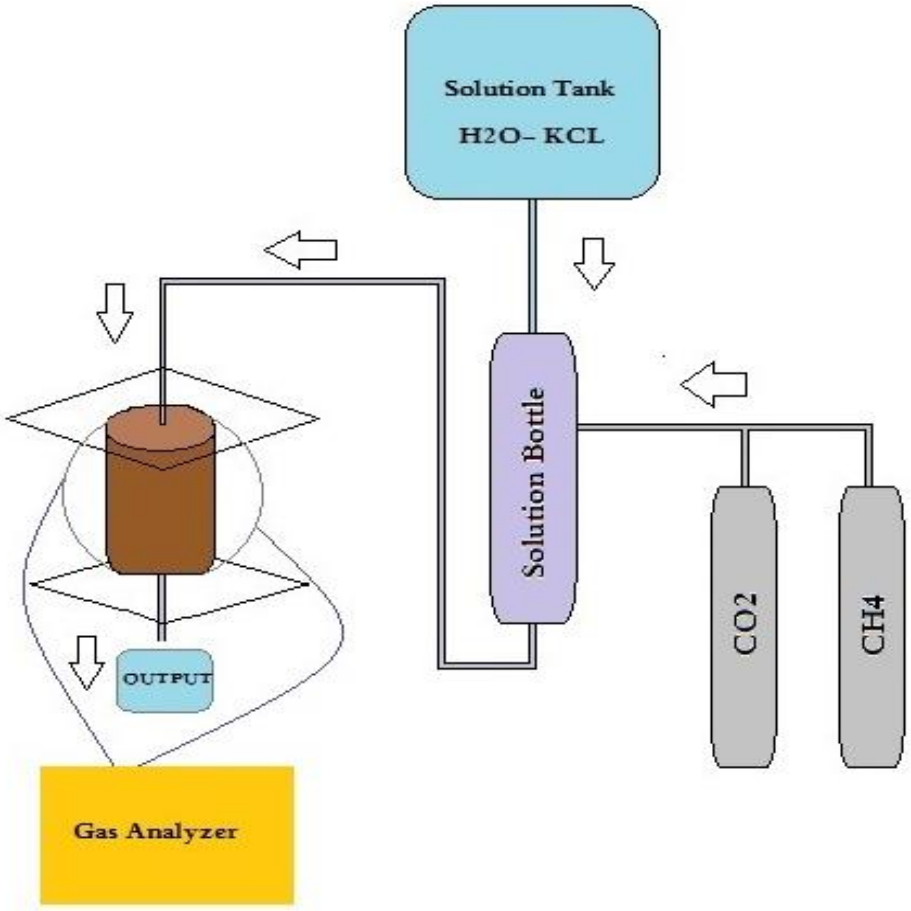

Figure 3 . Schematic of the Chamber set up

Two air circulating fans were embedded firmly in the body of the chamber. It will be necessary to circulate the air constantly, to prevent the accumulation of high concentrated gases inside the chamber. In addition, two valves were installed over the fans. To maintain the isolation of the chamber, valves were closed during the closure period. The chamber was connected to the analyzer by two acrylic extruded tubes (i.d.4mm). The inlet tube pumped sample gases into the analyzer and the outlet tube was connected from the analyzer back to the chamber. We used The Los Gatos Research (LGR) Ultra-Portable Greenhouse Gas Analyzer (UGGA) in this laboratory measurements. UGGA is an innovative ultraportable gas analyzer to measure methane, CO_2 and water vapor in one compact package. It can be operated in closed-loop setups with a flow rate of 0.245 $L\ min^{-1}$.

Figure 4. Conducting solution through the system by PVC tubing (A), Sealing the bottom and the top of the block by Neoprene rings (B)

3.3 Experimental protocol

The experiment was divided into two measurements: control and treatment. In the control measurement, the fluxes of the blocks and water flow were analyzed without methane and carbon dioxide addition to the flowing water, whereas in the treatment measurements, the fluxes and solution flows were analyzed from concentrations of methane and carbon dioxide in the flowing water-KCl mixture.

In each treatment (control, added-gases), five replicate chamber enclosures were measured. The chamber was closed for ten minutes in each enclosure. Two bottles of methane (5% CH_4 in 95% N_2) and carbon dioxide (99% CO_2, 1% N_2) were attached to the solution tank to inject the gases to the system continuously (Figure 3).

The first step in this process was to conduct CH_4 and CO_2 to a bubbling tank (Figure 4A) where both gases were expected to dissolve into the water-KCl solution. After bubbling in the tank, the solution was lead to the 20-liter container, from which the conducting solution was directed to flow to the top of the tree block.

3.4 Water sampling

We took samples of the input and output solution into 60 milliliter polypropylene syringes (BD Plastipak). Those samples were taken from the input solution before passing through the block and output solution after dripping from the bottom of the block. We repeated sampling for every five closures of the treatment with added methane and CO_2 dissolved into the solution water.

Prior to the analysis of samples , thirty milliliters of water was pushed out from the syringes and filled them with thirty milliliter of nitrogen (N_2). Then syringes were placed in 20^o centigrade water for thirty minutes and were equilibrated by shaking for three minutes.

Fifteen milliliter gas was taken from the equilibrated samples into the empty syringes. Contents of these syringes were injected to the analyzer directly. We applied nitrogen as the carrier gas (~700 – 800 $mL\ min^{-1}$) to reinforce the flow rate of sampling gases into the analyzer.

3.5 Chamber flux calculation

The chamber flux was assessed as the change of the sampling gas concentration (ppm) over the closure time (hour) and the measuring area of the chamber (m^2). The linear and exponential regression calculated by a MatLab-R2010a script (The MathWorks Inc., Natick, MA, USA) to compute the fluxes of methane and CO_2.

The equation estimated the flux value (F_0) during the closure time as

$$F_0 = S \frac{V}{A} \frac{M}{V_m} \frac{273.16}{273.16+T} 3600 \qquad [\ \mu g\, m^{-2}\ h^{-1}\ ]$$

Where S is slope of the appropriate fit ($ppm\ s^{-1}$)(In this study the linear regression [S_{lin}]), V the volume of the chamber (m^3), A surface area of the chamber (m^2), M the molecular mass of the element (CH_4= 16.42 , CO_2 = 44.01 [g mol^{-1}]), V_m the mole volume of the ideal gas (0.0224 $m^3 mol^{-1}$) and T the temperature of the chamber ($°C$) (Pihlatie et al. ,2013).

The outlier results of the chamber fluxes might result from sampling errors, leaking of the chamber and inevitable concentration fluctuations of the injected gases to the solution. Goodness of fit parameters including the normalized root-mean-square-error (NRMSE) and R-squared (R^2) were used to define the filtering criteria of the chamber fluxes. Any sampling result with R^2 less than 0.6 ($R^2 < 0.6$) and NRMSE more than 0.1 (NRMSE > 0.1), were removed from the data set. 20 data points were discarded by applying these criteria which belonged to the pine samples.

3.6 Calculation of diffusivity

This experiment also addresses the diffusivity of the methane and CO_2 through the sampling tree. Fick's laws of diffusion describe the macroscopic gas transportation in the material. The assumption is based on the effective diffusion process driven by concentration gradients (Tri Ho et al., 2006). Fick's first law of diffusion explained that diffusion flux and concentration gradient are proportional (e.g. Thompson, 2017). Particles under thermal movements flow from the higher concentration to lower concentration regions.

Here, the concept of diffusion flux (*J*) has been applied to relate the diffusivity of the gases through the body of the sample (*D*), measured flux (*F*), production (control condition flux) and internal changes of the sampling block (*ΔC*).

$$\Delta C = J_{in} - J_{out} - F + Production \quad (1)$$

where,

$$F = D \frac{C_{in} + C_{out}}{2} \quad (2)$$

C_{in} and C_{out} refer to the concentration of dissolved gas in the solution sampled from the top (C_{in}) and bottom (C_{out}) of the sampling block, respectively, J_{in} and J_{out} are fluxes of the solution at the top and bottom of the sampling block (Figure 5) where,

$$J_{in} = C_{in} \; V \qquad [\frac{mole}{s}] \quad (3)$$

and

$$J_{out} = C_{out} V \qquad [\frac{mole}{s}] \quad (4)$$

where $V(m^3 s^{-1})$ is volumetric flow rate calculated by measuring the volume of the flowing solution from the sampling block during the closure time (ten minutes). For this frame-work, considering the unit of parameters is a significant principle.

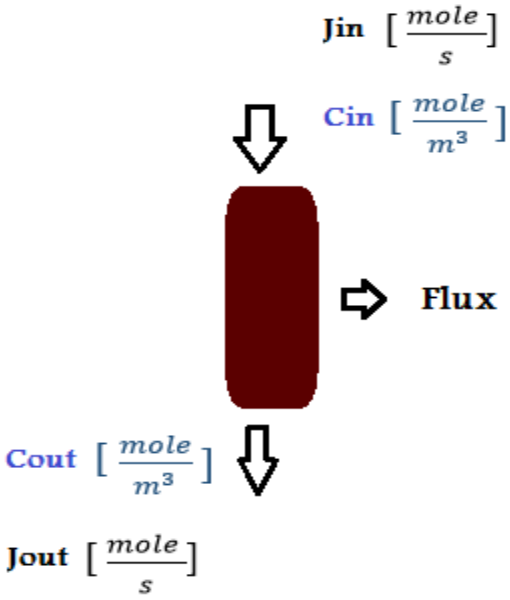

Figure 5 . Schematics of the position of factors (C_{in} , J_{in} ,C_{out} , J_{out}, Flux [F_0]) involved in calculating gas diffusivity. Arrows at top and bottom show the direction of input solution to the block.

As previously stated, C_{in} and C_{out} are the concentration of dissolved gas into the conducting solution taken from the top and bottom of the sampling block. Prior to calculating diffusivity, the concentration of dissolved gas needs to be calculated. The analyzer measured gas concentration values in the gas phase. Therefore, the measured gas phase methane and CO_2 concentrations in samples collected from the water (as explained in 3.4) were converted to the initial situation that the given gas was dissolved into the solution in the aqueous phase. Also the sample concentration readings of the gas analyzer (UGGA) were corrected by comparing the sample air to four known standards injected to the analyzer.

The formula for correcting the methane and CO_2 concentrations is demonstrated in Table 3. In the following regulations "C" refers to the concentration of the gas which has been obtained from the analyzer.

Table 3. Formulating the correction of the analyzer outcome for water samples concentrations.

Gas	Correction
CH_4	(5.8692 × C) – 4.36547
CO_2	(5.520199 × C) – 124.091

According to Henry's law, the amount of dissolved gas is proportional by Henry's law constant to the partial pressure of the gas phase at the constant temperature (e.g. Sander, 2015). Here, we used the dimensionless Henry solubility constant (H^{cc}) to define the ratio between gas phase and aqueous phase of the dissolved methane and CO_2 into the conducting solution.

$$H^{cc} = H^{cp}RT$$

Where R is gas constant (= 8.314 $molem^{-3}Pa^{-1}$), T is the temperature (room temperature= 293.15°K) and H^{cp} is Henry's solubility (1.4E-5 $molem^{-3}Pa^{-1}$)(adapted from Sander,2015). The H^{cc} was applied to direct the concentration of the dissolved given gases from the gas phase to the aqueous phase.

$$c_{aq} = H^{cc}c_{gas}$$

The concentration in the aqueous phase (c_{aq} [ppm]) were used to calculate the diffusion of methane and CO_2 and further related analysis.

3.7 Calculation of the water content

To estimate the water content of the test blocks, a fresh sample of each block was taken and restored in the laboratory freezer (– 20°C). At the end of experiments, all of the frozen drilled samples were put in a paper bag separately and dried in the oven (+ 60 ° C) for 72 hours.

The fresh weight and dry weight of the drilled samples were used to calculate the water content by following equation:

$$Water\ content = \frac{W_f - W_d}{W_f} \qquad [gr] \qquad (5)$$

Where W_f is fresh weight of the drilled sample and W_d is the dry weight of the sample after drying in the oven.

4. Results

4.1 Control measurement

The first set of plots provides an overview of the methane and CO_2 fluxes in the control condition for birch and pine samples (Figure 6). Each plot illustrates the CH_4 and CO_2 fluxes through the trunk from three different heights of an independent sampling tree. The closure numbers report the number of replicates for each height sampling.

A closer inspection of the plots shows the dissimilarity between pines and birches in the methane emission from the trunk. Although a significant emission was not observed in the control state, birches demonstrate a higher average methane fluxes compared with pines in control state (Figure 8 & 9).

Table 4. Comparing average flux values for sampling species in control condition

Species	$\overline{\text{Flux}}\ CH_4$ $[molem^{-2}s^{-1}]$	$\overline{\text{Flux}}\ CO_2$ $[molem^{-2}s^{-1}]$
Pine samples	0.2×10^{-10}	0.8×10^{-6}
Birch samples	3×10^{-10}	3×10^{-6}

The height of the sample does not influence the methane and CO_2 fluxes significantly. There were no standard pattern considering the effect of sampling height on the measured fluxes. It is apparent from these plots that a few of the samples reached steady state during the measurement, but a few of the control samples show a slight fluctuating in flux values under the control conditions.

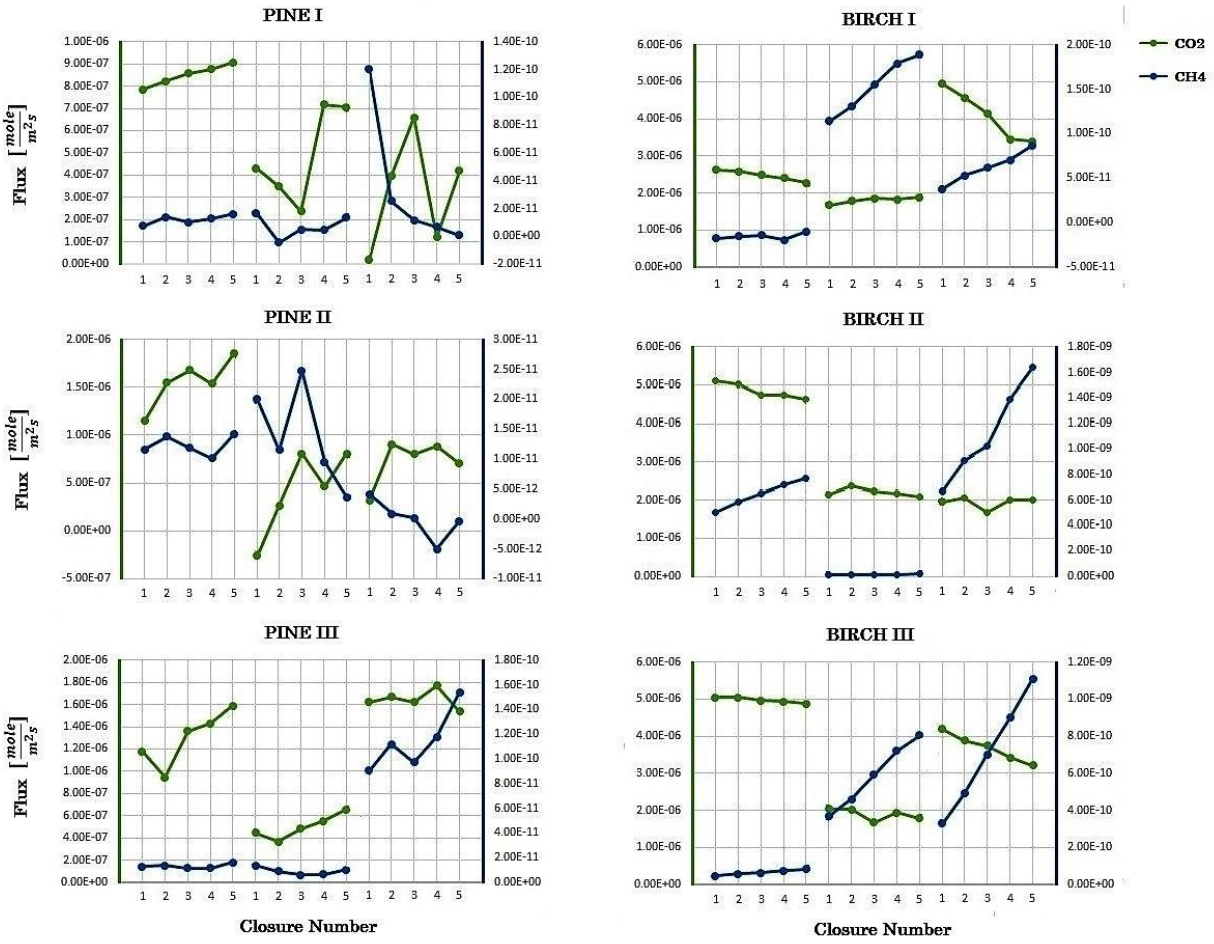

Figure 6. Methane (-o-) and CO_2 (-o-) fluxes though the tree trunk for sampling pines (left) and birches (right) – Closure numbers from left to right (horizontal axis) refer to closure numbers of bottom, middle and top cuts from the tree height.

4.2 CH_4 and CO_2 added

Here, the results indicated the methane and co2 fluxes of samples after the treatment. In the treatment measurements, methane and CO_2 were injected in the flowing water-KCl mixture. From the data on Figure 7, it is apparent that birches had a higher flux value of methane and CO_2 compared to pines from the tree trunk. The average flux of the methane for birch samples is considerably higher than the average radial methane fluxes for pine samples. Strikingly, the average radial flux value of the CO_2 in pine samples is in the same relative range with the average flux value of CO_2 in birch samples (Table 5). Although birch samples demonstrate a higher average methane fluxes compare with pine samples in total (Figure 8 & 9).

Table 5. Comparing average flux values for sampling species after adding methane and CO_2 gases

Species	$\overline{\text{Flux}}\ CO_2\ [molem^{-2}s^{-1}]$	$\overline{\text{Flux}}\ CH_4\ [molem^{-2}s^{-1}]$
Pine samples	1×10^{-6}	0.8×10^{-9}
Birch samples	4×10^{-6}	8×10^{-9}

Comparing the two species, it can be seen that methane fluxes and CO_2 fluxes are following the same trends in the majority of cases. Although pine samples demonstrate irregular methane emissions compared with birch samples, it is counterintuitive that their CO_2 emission trends seem regular. The inspection of the plots shows that in few instances the steady state was reached at the end of the experiment, indicated by a stabilized flux rate (Figure 8, pine III). Pines achieved steady state better compared with birches. Interestingly, CO_2 seemed to reach the equilibrium condition faster whereas methane fluxes remained to increase throughout the five replicate measurements.

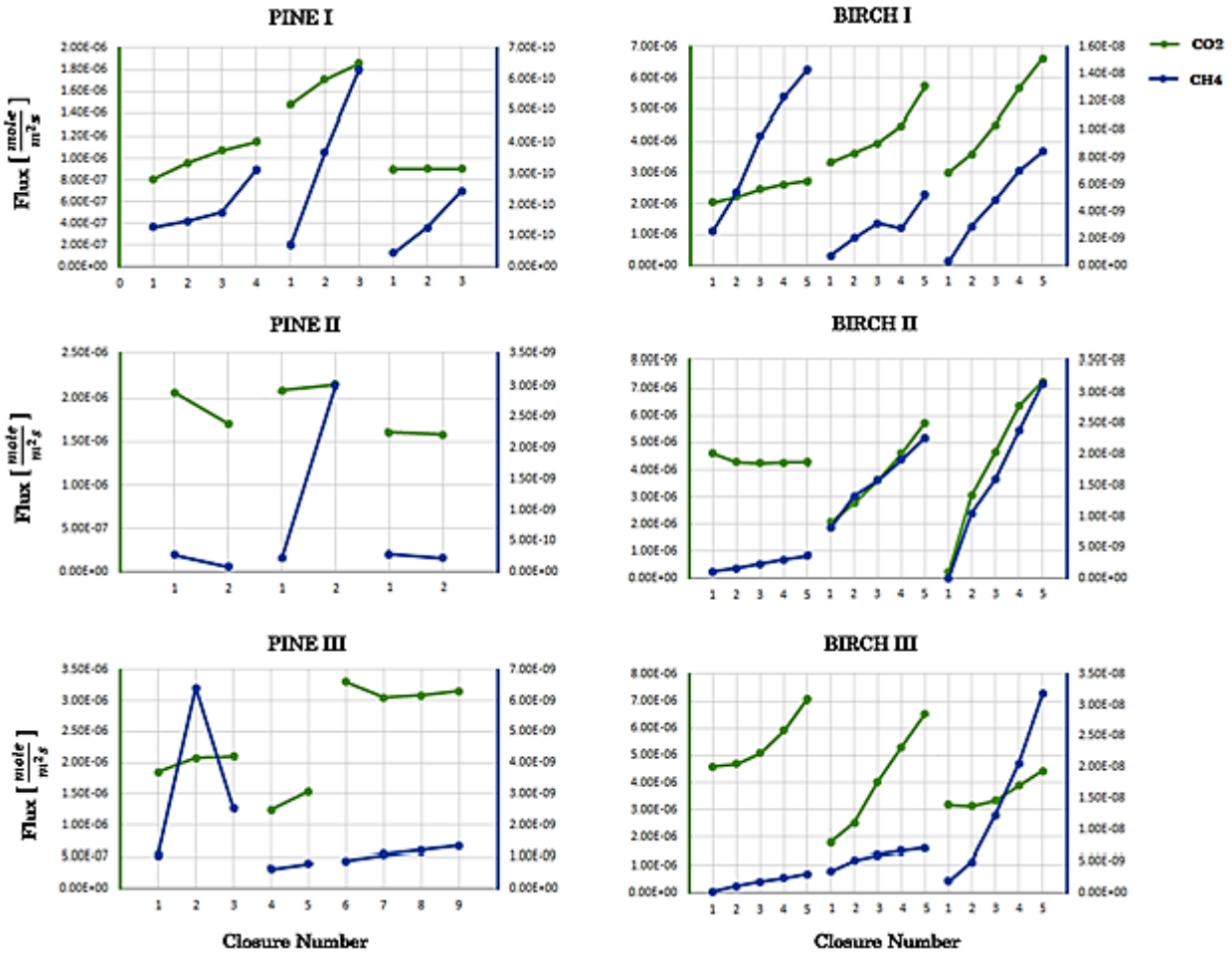

Figure 8. methane (-o-) and CO_2 (-o-) fluxes though the tree trunk for sampling pines (left) and birches (right) after adding methane and carbon dioxide gases to the conducting solution – Closure numbers from left to right (horizontal axis) refer to closure numbers of bottom, middle and top cuts from the tree height.

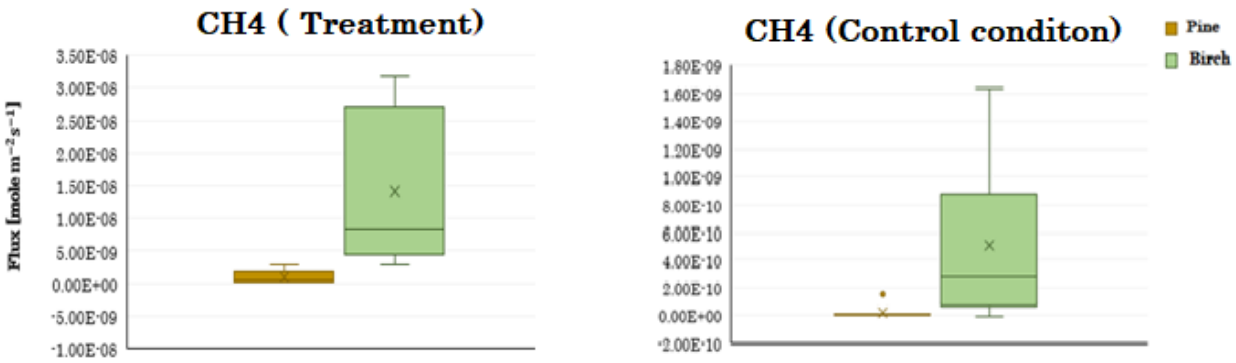

Figure 8. Boxplots for comparing mean CH_4 fluxes of all Pine and Birch samples after adding gases to the conducting solution (treatment) and control condition.

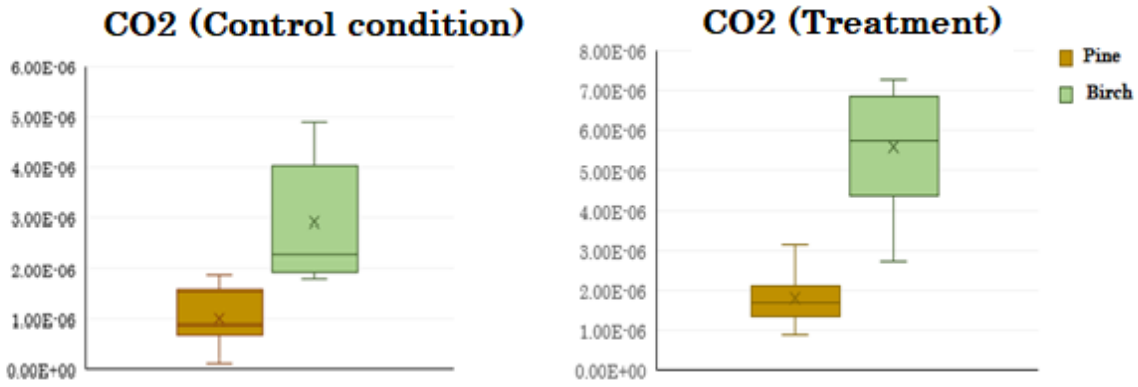

Figure 9. Boxplots for comparing mean CO_2 fluxes of all Pine and Birch samples after adding gases to the conducting solution (treatment) and control condition.

4.3 Diffusivity of CH_4 and CO_2

In order to assess diffusivity values, eq.2 was used. The obtained flux values and concentration of dissolved gas into the input (C_{in}) and output (C_{out}) solution were replaced in the equation to calculate the diffusivity of methane and CO_2 for the tested samples. Data for diffusivity calculation were collected using the average fluxes of two last chamber closures which are closest to the steady state. C_{in} and C_{out} values refer to the same closures. Table 6 demonstrates the average diffusivity values of methane and CO_2 for different heights of the tree trunk for birch and pine samples, separately.

In the table 6, the different heights were indicated by numeric figures I, II and III those refer to the bottom, the middle and top height of the tree, respectively. The differences between the average diffusivity of the methane for the birch samples and pine samples are highlighted in Table 6.

Birch samples showed higher values for methane diffusivity compared with the pine samples. What is striking about the data in the table 6 is the average diffusivity of the CO_2. The results show significant dissimilarity between the diffusivity of methane and the diffusivity of carbon dioxide for both species.

Figure 10 compares the average of methane and CO_2 diffusivity of various heights for birch and pine samples. As shown in the figure, the average diffusivity of both gases at different heights of birches is following the similar trends. The results from the pine average diffusivity do not demonstrate the same pattern.

Table 6. The average diffusivity values of Pine and birch samples, classified based on the height of the tree trunk.

Species and Height	$\overline{D}\,CH_4\,[\frac{mole}{m^3}]$	$\overline{D}\,CO_2\,[\frac{mole}{m^3}]$
Birch I	18×10^{-11}	40×10^{-6}
Birch II	3×10^{-11}	1×10^{-6}
Birch III	3×10^{-11}	$4x\ 10^{-6}$
Pine I	0.4×10^{-11}	0.8×10^{-6}
Pine II	0.3×10^{-11}	0.9×10^{-6}
Pine III	0.09×10^{-11}	1×10^{-6}

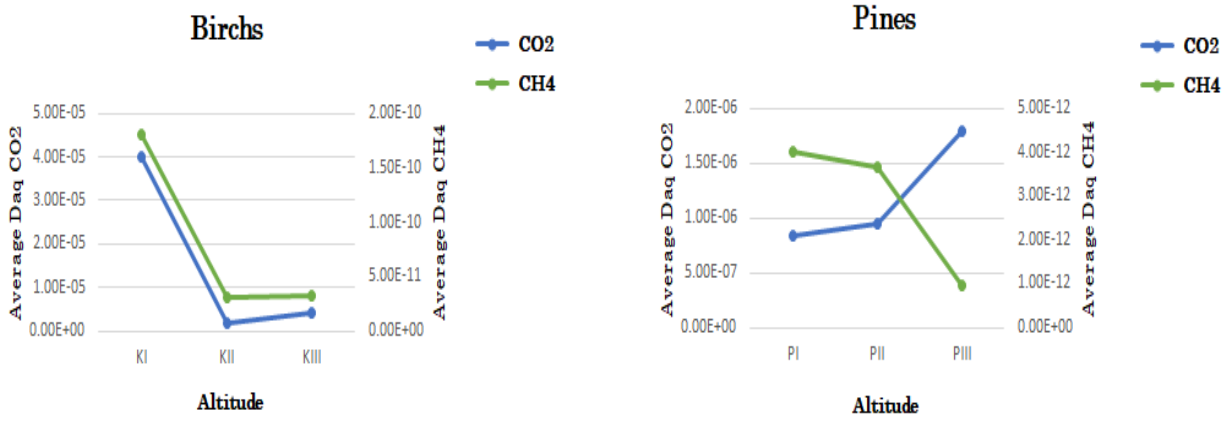

Figure 10. Average DCO2 Vs. Average DCH4 for birch and pine samples in various heights.

4.4 Water content and Diffusivity

The water contents (Eq.5) of different heights of the tree trunk are displayed in Figure 11. The number of heights were demonstrated as 1,2,3 for the bottom, the middle and top heights of the tree trunk, respectively. It can be seen from the figure that the pine samples have higher water contents compare with the birch samples. Birch samples do not show significant fluctuation for the water content in various tree trunk heights.

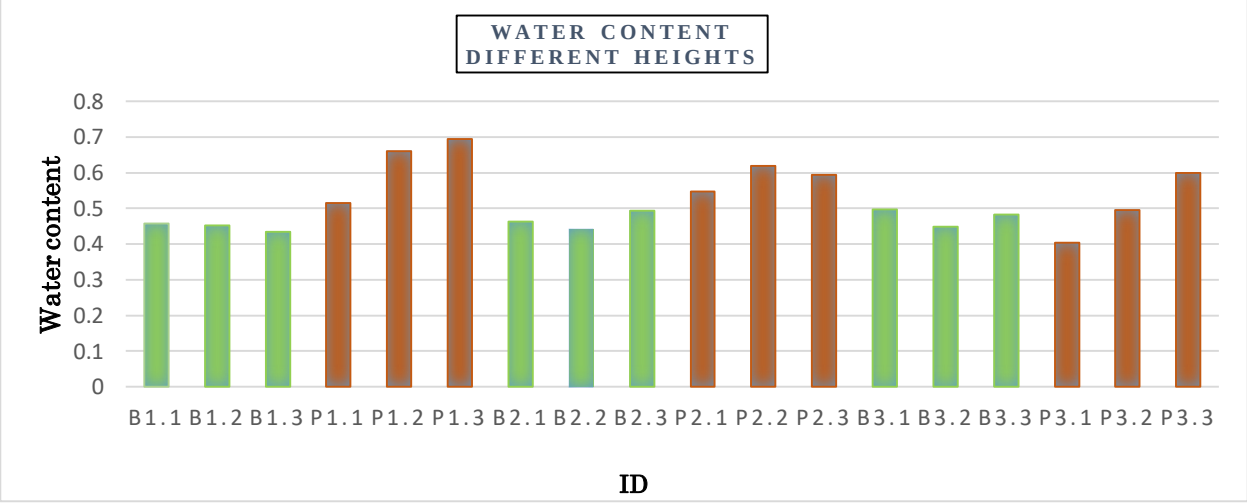

Figure 11. Water content (*gr*) of different height of all tested samples. Green bar demonstrates the birch samples, and brown bar demonstrated the pine samples. The digits in ID present the number of sample and number of height.

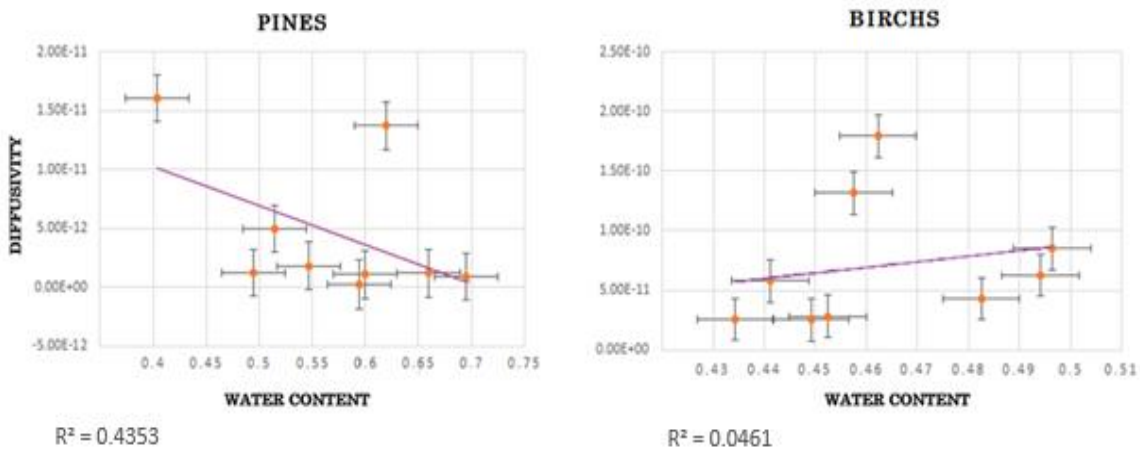

Figure 12. Average methane diffusivity ($\frac{mole}{m^3}$) Vs water content (gr) for Pine and Birch samples. The trend line and error bars were added to evaluate the linear regression of parameters.

The correlation between the diffusivity of methane and water content was tested in Figure 12. The water content estimated the porosity of the tree samples based on the correlation of the dry weight and fresh weight of the samples (Ekelman,2015). It is apparent from this figure that the pine samples illustrate the decreasing trend line. However, the birch samples show slightly increasing trend line to the similar parameters, but it is not significant.

4.5 Water Flow

The amount of solution which has been flowing through the sampling tree during the closure period defines the water flow rate. Figure 13 reviewed the sum of the water flow of the various heights for pine and birch samples.

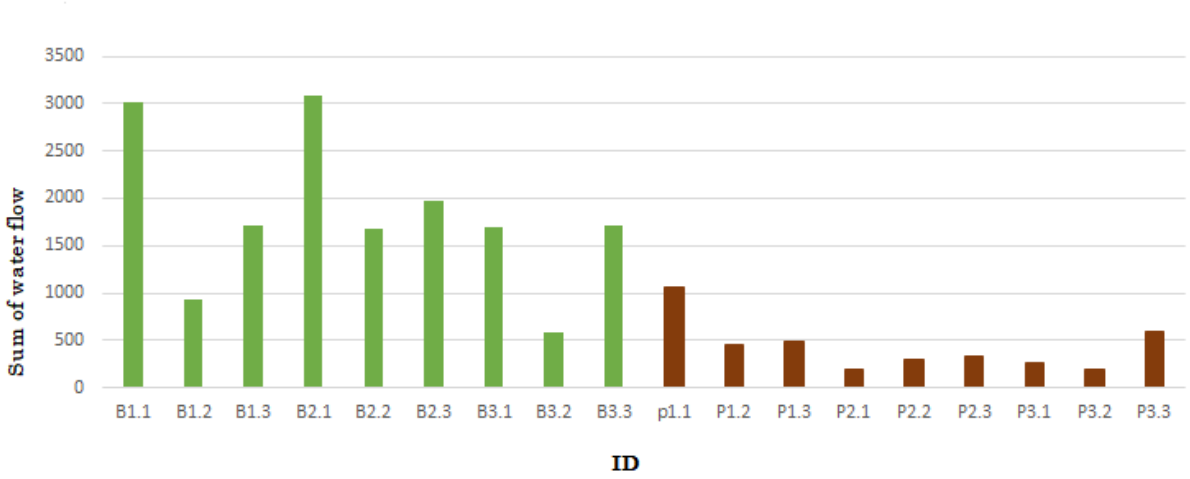

Figure 13. Comparison of the sum of the water flow for birch and pine samples. [mLiter per 10 min]

From the Figure 13. it is clear that the sum of the water flow for birch samples is considerably higher than pine samples. Water flowing in the stem of different trees indicates the differences in the hydraulic conductivity of species (Lintunen & Kalliokoski, 2010). The significant difference of water flow rate for pine and birch point out to their distinct hydraulic conductivity. Birch has higher hydraulic conductivity due to its larger water conducting conduits in the xylem (Lintunen & Kalliokoski 2010).

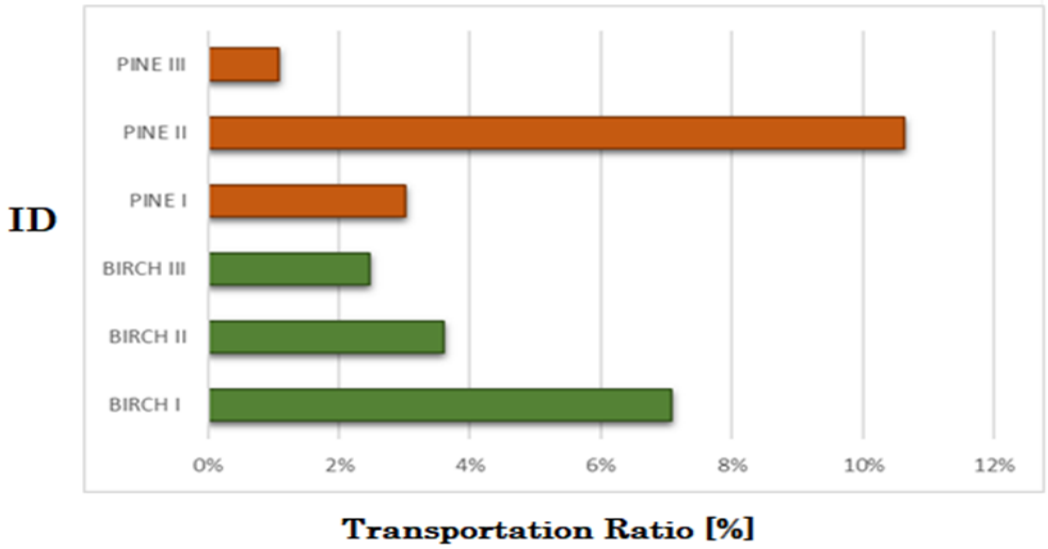

Figure 14. Vertical transport of methane through the tree stem in conducting solution, expressed as the ratio of J_{OUT}/J_{IN} .Where , J_{IN} and J_{OUT} are fluxes of the solution at the top and bottom of the sampling block.

4.6 Gas transportation ratio and Internal changes

Turning to the experimental evidence on the internal transport of gas through the stem, Figure 14 compares the transportation ratio of methane for the sampling tree species. The transportation ratio is obtained from the ratio of the $\frac{J_{out}}{J_{in}}$, $(\frac{Eq.4}{Eq.3})$,to assess the amount of gas which passes through the stem.

Pine samples demonstrate the highest and lowest transportation ratio among two species by under 2% and above 10%, respectively. Birch samples provide the average transportation ratio about 4%. It is apparent that only few percentage of the methane transported with water (2-10%), and most of the methane within tree stem (up to 98%), was transported via radial diffusion and emitted through the stem (flux).

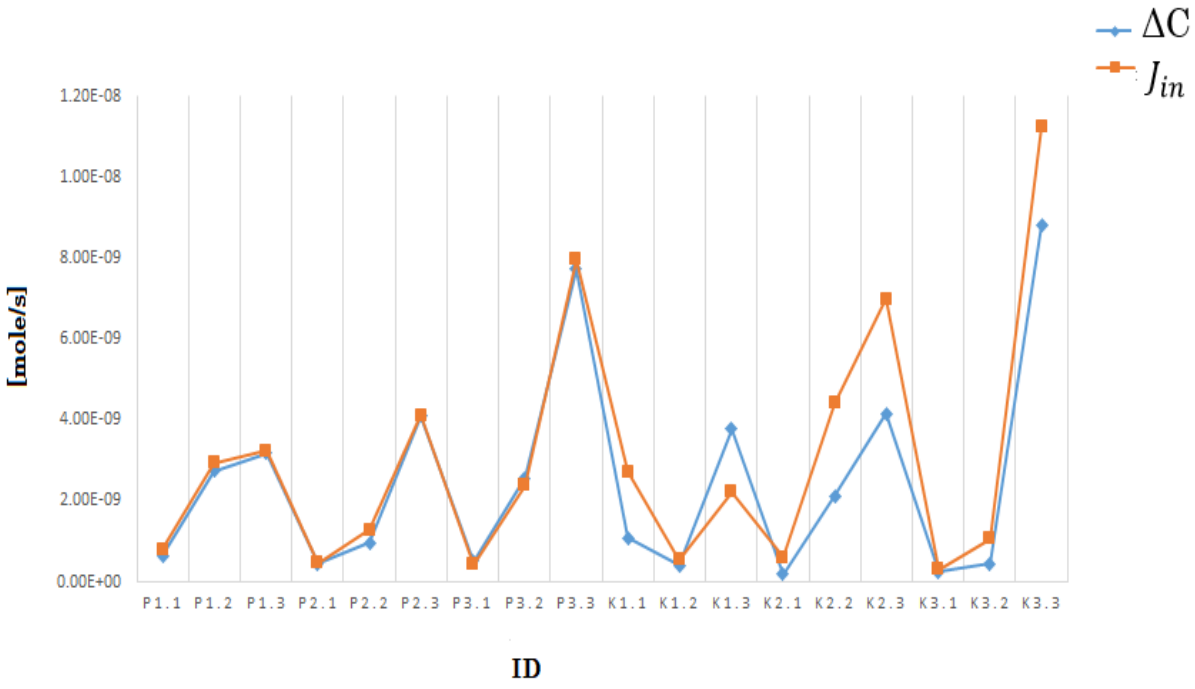

Figure 15. Comparison of ΔC (eq.1) with J_{in} (flux of input solution)

(P stands for pine and K stands for birch)

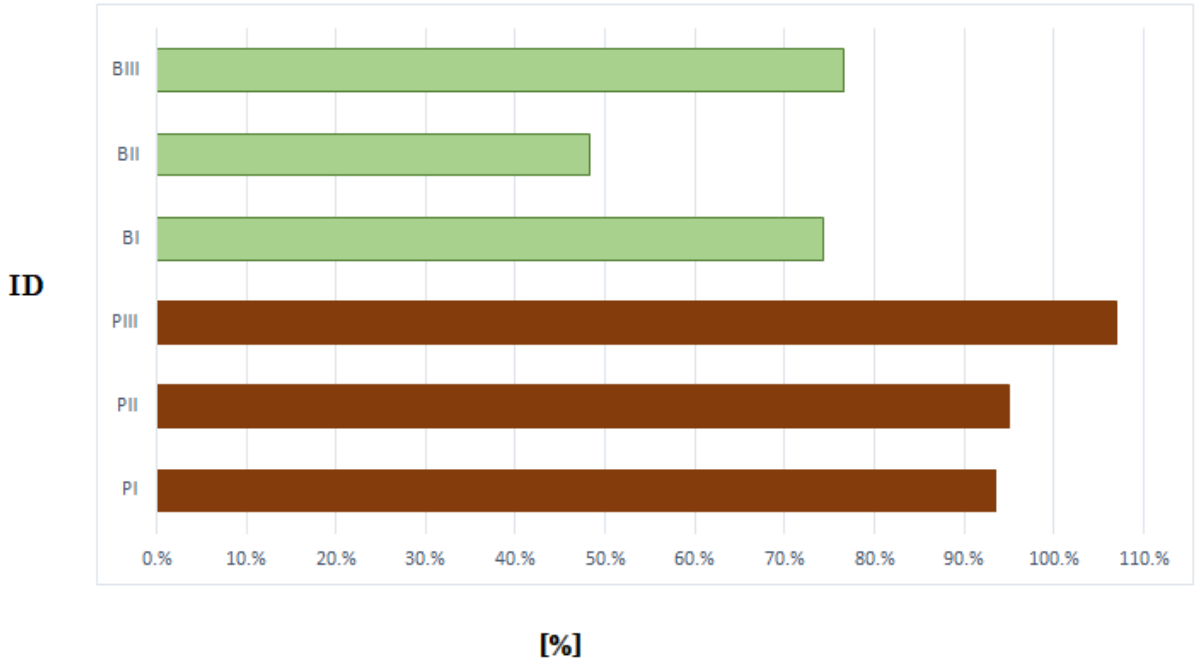

Figure 16. The amount of methane accumulated inside stem for birch (B) and pine (P) samples.

Figure 15 demonstrates a comparison between ΔC (eq.1) and J_{in} (eq.3). Figure 16 shows the ratio of the methane content inside the stem to the total input gas through the stem. These values obtained by estimating the median of the ratios for different heights of each sampling tree. The results lead to a conclusion that larger amounts of methane accumulated inside the stem in pine samples. Figure 15 shows the same result by comparing the input methane fluxes with internal changes.

5. Discussion

5.1 CH_4 and CO_2 flux, laboratory measurements and field experiments

This study set out with the aim of assessing the importance of boreal forests in the global methane budget. This project aims to develop an overarching framework to evaluate the methane transport capacity of tree stem and wood anatomy difference of dissimilar species in the transporting capacity.

Previous studies have noted the effect of trees on the emitted methane from the forests. Machacova et al. (2016) showed that Scot pines are one of the missing sources of nitrous oxide (N_2O) and methane in the boreal forests. Their studies indicated that median value of emitted methane from the tree stems is 0.005 $\mu g m^{-2} h^{-1}$.

Pangala et al. (2015) have highlighted the significant methane release from the stem of Betula pubescens and *Alnus glutinosa* during their field experiments. Their study highlighted the seasonal variation of fluxes for dissimilar species. It determined that the highest stem flux of *Betula pubescens* was 203±21 $\mu g m^{-2} h^{-1}$ during the summer. Also Pangala et al. (2017) published results of studying Amazonian floodplain tree stems from the central amazon basin. The results reported that floodplain tree emit 15.1±1.8 to 21.5±2.5 teragrams methane per year. Young and mature trees emit 0.39–581 $mg\ m^{-2} h^{-1}$ and 0.33–337 $mg\ m^{-2} h^{-1}$ methane per unit stem surface, respectively.

This laboratory framework concluded the average methane stem flux of Scot pine samples in the control state was 0.66 $\mu g m^{-2} h^{-1}$ (= 2.38E-11 $mole m^{-2} s^{-1}$) and the average methane stem flux of *Betula pubescens* samples in the control state was 21.48 $\mu g m^{-2} h^{-1}$ (= 3.71E-10 $mole m^{-2} s^{-1}$). The results revealed that the birch samples have a higher average stem flux compare with pine sample which supports the finding on the previous unpublished field measurements at boreal forest sites (personal communication, Mari Pihlatie).

The average methane stem flux of pine and birch samples after injecting CO_2 and CH_4 into the conducting solution were 51.34 and 465 $\mu g m^{-2} h^{-1}$, respectively. The difference of the average fluxes for dissimilar species and the flow rate of the various samples (Figure 13) provide evidence that the anatomical disparity between two species will influence the transport capacity of their stems.

In line with previous studies, the findings from Machacova et al. (2016) and Pangala et al. (2015,2017) are focusing on sampling of different locations. Pangala et al. (2015) mentioned that the higher methane fluxes could be due to higher temperature and the higher soil methane production rate. The average summer temperature of Pangala's sampling site is mentioned as 15.5°C while the measurements in Machacova et al. (2016) paper were conducted in Finland where the annual average temperature of the sampling location is 3.5°C. In our laboratory study, the temperature is not considered as the ruling parameter.

It is important to note that most of the stem CO_2 originates from the respiring cells in the stem and roots (Teskey et al., 2008). Studies (Pruyn et al,2003), (Hölttä & Kolari,2009) emphasized that CO_2 efflux from the tree is strongly related to the sap flow rate, geometry and size of the tree stem.

Here, the differences of average values for CO_2 fluxes for pine and birch samples ties well with the previous studies. However, the CO_2 results could not answer the research questions about the stem diffusivity, as CO_2 production by respiration was very large in comparison to CO_2 fed to the tree. This problem could possibly have dealt by killing the living cells in the stem by, e.g. a heat treatment, so that respiration would cease.

5.2 What do we know of CH_4 and CO_2 diffusivity in wood

In the field experiments, the tissue size of the tree and seasonal cycle are the dominating parameters to evaluate the xylem diffusion and the respiring cell diffusion of the CO_2. The stem conducts the trapped carbon dioxide to the internal recycling process and utilizes it in the green woody tissue or in the leaves in photosynthesis (Aschen and Pfanz, 2003).

As previously stated, the wood anatomy of the tree affects the transport and diffusion of the gas within the stem. Pangala et al 2014 stated that the variation of methane concentration in flooded A. glutinosa samples is explainable by lenticel density and methane concentration of the pore water. Wang et al. (2017) have mentioned stem water content as the dominating factor of the gas diffusion rate. Rusch and Rennenberg (1998) highlighted that the diffusion drives methane through the stem of the wetland adapted *Alnus glutinosa* samples.

In this framework, studying the diffusivity of two different sampling trees within the stem suggested that birch samples have higher average methane and carbon dioxide diffusivity values compare with the pine samples (Table 6). This result may be explained by the fact that the anatomical composition of species, including heartwood, sapwood, bark tissue and lenticel densities are different. There are, however, other conceivable explanations including water content, the water flow levels and possible internal microorganism activities.

One unanticipated finding was that the stem flux rates of pine samples in control treatment ties well with previous the stem flux measurements in the field wherein higher water content proceeds as the diffusion barrier (Figure 12). Although the birch samples are not in accordance with stated findings, but pine samples demonstrate the higher water content in total value (Figure 11) compared with birch samples. This result reflects those of Wang et al. (2017) who also mentioned the inverse correlation between water content and diffusion rate. The lower water content of the birch would cause the longer time to reach the steady state due to the more air spaces to be filled with air. Therefore, the same results would have been concluded for birches if they also reached the steady state.

5.3 Methane radial transportation and transpiration stream

An initial objective of this project was to evaluate the methane transport capacity of tree stems for the selected tree species. This study concluded that birch samples release higher amounts of methane through the stem compared with pine samples both in control conditions and after adding methane to the conducting solution (Table 5). The results highlighted that the average methane fluxes of birch samples in the control condition (Figure 7) and their average methane fluxes after adding the gases to the conducting solution (Figure 9) are higher compared with the pine samples under the analogous treatments.

Figure 13 presented an overview of the water flow level for various birch and pine samples during the closure period. The figure confirms that birch samples provided higher water flow rate compared with pine samples. Birches demonstrate a higher accumulated methane inside the stem (Figure 16). As it stated previously, methane does not solve in the water and rather diffuses through the air space. Here, results could conclude that transpiration stream is not important in the methane transport. Also, the higher diffusivity in birches and air porosity led to the higher methane fluxes through the tree stems. There are, however, other possible explanations including the anatomical composition of the tree and possible internal microorganism activities.

Figure 14 provides information regarding the transportation ratio of the methane through the stem. it is clear that most of the methane within the tree stem, is emitted through the stems as flux. In general, therefore, it seems that pine samples transported a larger portion of gas through the stem, vertically. Pines also accumulated larger amount of methane inside the stem and showed lower water flow rate, respectively (Figure 16). Birch samples transported about the 4% of the methane on average through the similar pathway.

5.4 Conclusion and Future improvements

This study has shown that birch samples have higher methane stem fluxes compared with pine samples under control conditions and in methane and CO_2 addition treatments. The finding on the previous experiments supports the test results. The result also indicated that birches accumulated less methane inside the stem compared with pine samples.

One of the more significant findings to emerge from this study is that birch samples have the higher average methane and CO_2 diffusivity within the stem compared with pine samples which may be explained by the dissimilarity of the anatomical composition of species, including heartwood, sapwood, bark tissue and lenticel densities.

Future research should consider the potential effects of the methane insolubility in water more carefully. A closed solution container with zero possibility for methane to run away the solution might be applied to add the constant methane and carbon dioxide to the solution, before every closure. The input methane concentration should be closer to the methane concentration in the natural condition to avoid unnecessary difficulties including bubble formation in the system. This method might provide better results to measure the transportation ratio and diffusivity values by accessing the initial concentration of the injected gas to the solution.

It will be important that future research considers the longer measurement period. Measurement should be conducted long enough that methane flux reaches the steady state. This is the desirable result which should be followed up for the future.

Another assumption to address in future studies could be improving the output water sampling method. A direct tubing connected to the vial during the closure could measure the water flow rate. The same vial can be used to estimate the output gases concentration. The more accurate sampling method would help to establish a greater degree of accuracy on the modeling flux and diffusivity of the tree stem in this study.

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